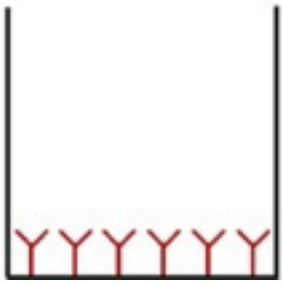
Neogen Veratox Method for DON

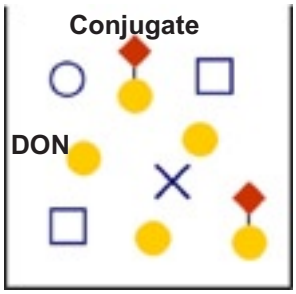
Overview. Neogen's "Veratox" (DON) uses ELISA technology. Antibodies specific for a mycotoxin are adhered to the inside of a microwell.

Figure 6. Neogen Microwell.



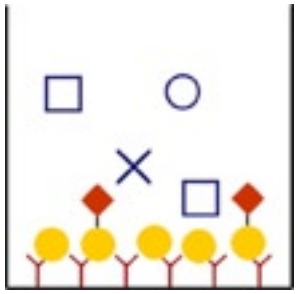
A solution of DON, chemically conjugated to an enzyme, is provided with the kit. A sample to be tested for DON is ground and extracted. The extract is then filtered and mixed with a fixed amount of the DON-enzyme conjugate solution in a mixing well.

Figure 7. Mixing well containing extract and conjugate.



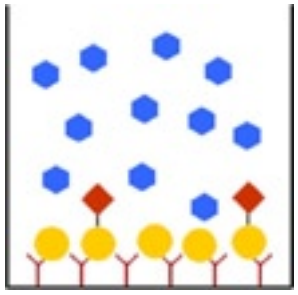
A portion of the mixture is then transferred to the antibody well. The DON from the extracted sample and DON-enzyme conjugate then compete for the antibody binding sites in the microwell.

Figure 8. Free toxin and conjugate compete for binding sites in microwell containing antibodies.



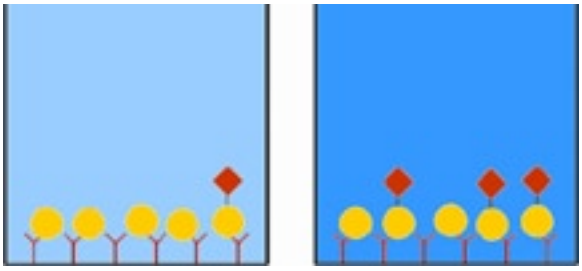
The assay procedure measures how much of the conjugate actually binds to the antibodies by first thoroughly washing the microwell then adding a colorless substrate.

Figure 9. After washing substrate is added.



The enzyme present in the microwell converts the substrate to a blue colored product; the more DON-enzyme conjugate in the microwell, the more intense the blue color.

Figure 10. The conjugated enyme and substrate react to form a blue color.



Because samples with high DON will result in less binding of the DON- enzyme conjugate, positive samples will be lighter blue. Quantitative measurements are obtained by measuring the intensity of the color with an optical density reader.

Veratox Procedures. The Neogen Veratox DON test is provided as a kit containing all required reagents, controls and microwells.

Photo 5. Neogen Veratox test components.

NOTE: When not in use, kits should be stored in the dark at refrigeration temperatures. Prior to use, all necessary kit components must be equilibrated to room temperature. The use of cold reagents could adversely affect the color development of the ELISA test.

The kit components are as follows:

-Foil pouch with Antibody-coated well strips and red marked mixing well strips.

-Substrate. The substrate is pre-activated and ready for use.

-Conjugate. Unseal one of the conjugate bottles and remove the rubber stopper. Cut the tip off the enclosed squeeze tube. Squeeze tube contents into the bottle. Replace the stopper and swirl, don't shake, contents until the pellet has dissolved. Use the contents of the bottle until empty.

NOTE: Once rehydrated, contents must be used within 3 weeks. Mix the second bottle of conjugate in the same manner when needed. Refrigerate when not in use.

-Controls. The kit comes with 6 controls (0, 0.5, 1, 2, 4, and 6 ppm). The 4ppm control is not used in the official procedure.

-Red Stop. Swirl to mix prior to use.

TIP: Swirl, don't shake all reagents prior to use so as to mix them but not cause them to foam, which could cause pipetting errors later.

Step 1: Thoroughly mix the ground sample and weigh out 50 grams. Place the ground sample in an 18 ounce nasco whirlpack bag or clean plastic or glass container.

Step 2: Add 250 ml of distilled or deionized water. **Do not use tap water!** The pH of tap water may adversely effect results.

Photo 6. Adding distilled water.

Step 3: Shake mechanically or by hand for 3 minutes. Let the material stand for 1-2 minutes to let some of the sample settle.

Step 4: Place a sheet of filter paper (Whatman #1 folded or equivalent) into a clean funnel mounted over a test tube or collection beaker. After much of the sample slurry has settled to the bottom of the bag, pour at least 15 mL of the extract through the filter paper.

Photo 7. Filter Extract.

Step 5: Remove a red-marked mixing well strip and break off the needed number of wells-one for each control and one well for each sample (up to seven). Return unused strips to package.

Remove an antibody-coated well strip and break off the same number of wells. Mark one end of the antibody-well strip with a 0 (zero) for the blank and the other end with an S for samples and place strip in the well holder with the 0 marked end on the left.

NOTE: Do not mark the inside or bottom of the antibody wells.

Step 6: Firmly place a pipette tip on the 100µL pipettor and add 100µL conjugate to each mixing well. Discard tip.

Photo 8. Add conjugate to each mixing well.

TIP: Prime pipette tips before dispensing conjugate. To prime a tip, draw up some of the reagent to be dispensed and discharge it back into the same container. Priming coats the inside of the pipette tip so that the volume dispensed will be identical during repeated use of the same tip.

Step 7: Remove the stopper from the 0 ppm control bottle. Using a new pipette tip, add 100µL of the 0 ppm control to the first mixing well (labeled 0). Discard tip and replace cap on control bottle. Repeat with the 0.5, 1.0, 2.0, & 6.0 ppm control bottles, using a clean tip for each control.

Add 100µL of filtrate from the sample collection tube of the first sample to the sixth well. Discard tip. Repeat for each sample, placing 100µL from each in a different well. Use a new tip for each sample.

TIP: When drawing reagents into a pipette tip, drag the tip across the rim of the reagent bottle to remove any excess liquid.

TIP: When dispensing reagents into the microwells, place the tip point against the inside wall of the microwell. This helps draw all of the liquid out of the tip and eliminates drops that form on the end of the tip.

TIP: Always check the fluid levels in your tips prior to dispensing to be sure that the same amount is being collected each time. If the proper amount was not collected, or bubbles are present, refill the tip.

Step 8: Firmly place pipette tips onto the 12-channel pipettor. Mix the solutions by depressing the plunger 5 times. Transfer the solutions to the marked antibody wells. Discard the red marked mixing wells.

Photo 9. 12-channel pipettor.

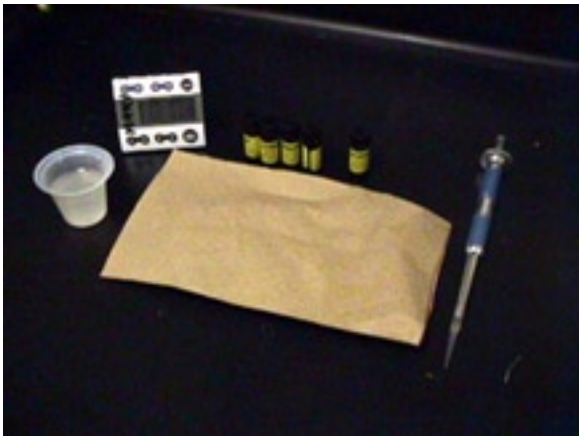
Step 9: Mix by sliding the Microwell holder back and forth on a flat surface to ensure mixing (15-20 seconds). Do not splash reagents from wells.

Step 10: Cover the wells to protect them from dust and allow them to incubate for 10 minutes.

Photo 10. Mix solutions.

During this incubation, the conjugated DON and any free DON from the sample or standard will compete for binding sites in the antibody wells. The more free DON in solution, the less conjugate will be bound to the antibody well.

Photo 11. Cover the wells during incubation.



Step 11: Initial reaction is now complete. Shake out the contents of antibody-coated wells. Using a wash bottle, fill each antibody-coated well with distilled water and shake out. Repeat five times. Any bound conjugate or DON will not be removed during the wash steps.

Step 12: Remove all large water droplets by turning wells upside down and vigorously tapping wells on paper towel. Bound conjugate or DON will not be removed by the tapping.

TIP: To remove water droplets, wrap the well holder in a paper towel and rap the wells vigorously five or six times or until no fresh water droplets appear on the paper towel. When the water has been removed, loosen the wells in the well holder so they will be easier to remove after the substrate and red stop have been added.

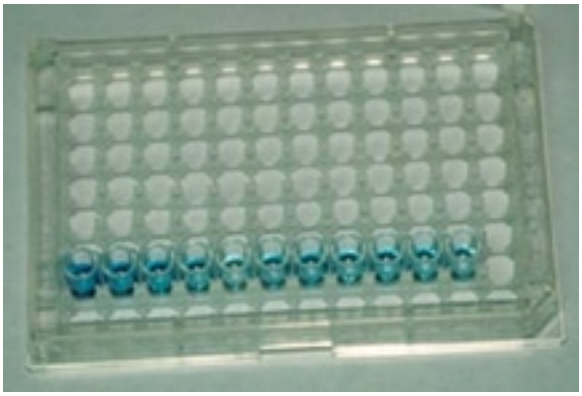
Step 13: Pipette 3 mL of substrate into reagent boat and, with new tips on the 12-channel pipettor, place 100µL substrate into the wells.

Step 14: Mix by sliding wells back and forth on flat surface. Cover the wells to protect them from dust. Incubate 10 minutes.

Photo 12. Removing water droplets from wells.

During the 2nd incubation, color development will begin. The substrate will react with the DON-conjugated enzyme to change the reagent color to blue. Less toxin bound means more conjugate present, and the darker the blue color.

Photo 13. Color development.



Step 15: Pipette 3 mL of red stop solution into a clean reagent boat. Using the 12-channel pipettor add 100µL of red stop to each well and mix as before. This will halt the color development.

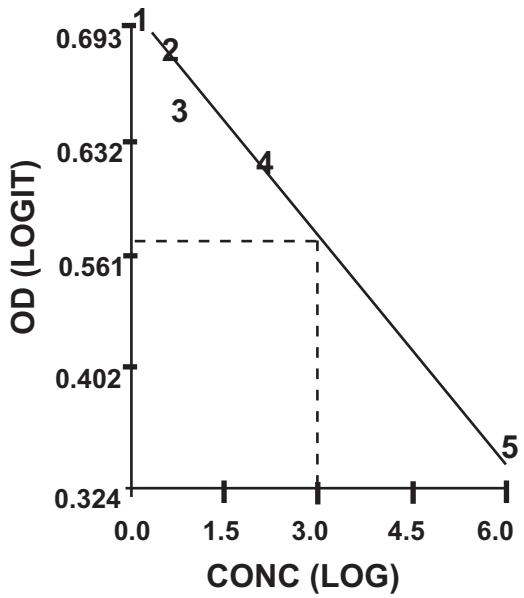
Step 16: The optical density (amount of color) of each well is read on a microwell reader. The optical density (OD) values of the controls are entered into the Log/Logit program used to develop a calibration curve.

Photo 14. EL 301 microwell reader.

NOTE: Neogen also offers an automated microwell reader (Stat Fax 303 Plus). Contact your Neogen representative for information.

TIP: For optimum results the OD on the zero standard should be 0.6 or higher. Cool laboratory temperatures (below 72 F) may slow the reactions requiring a longer incubation period. If your OD on the zero standard is less than 0.6 try lengthening the incubation time to 11 or 12 minutes.

Figure 11. Veratox calibration



The Log/Logit program provided by Neogen first normalizes the data to create a linear (straight line) relationship. It then performs a linear regression to determine the straightline that best fits the control data.

The program reports two statistical values which are used to determine if the calibration is acceptable. The **correlation coefficient** values, or "r", indicate how well the five control samples fit the line. If all five of the values fall exactly on the line the correlation is perfect (1.0). The correlation must read 0.98 or higher to ensure accurate results. If correlation value is less than 0.98, rerun the test.

The **slope** value indicates how steep the line is angled. The slope value must read –2.0, +/- 0.5.

NOTE: Do not certify results if the correlation coefficient is less than 0.98 or the slope value is out of tolerance. If the slope value consistently reads outside these tolerances, or if the correlation coefficient is consistently below 0.98 call Neogen (1-800-234-5333) for troubleshooting assistance .